In vitro EBV Infection of Mononuclear Cells that Have Been Cryo-preserved

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[Abstract] Epstein-Barr Virus (EBV) is a B-lymphotropic herpesvirus which the majority of adult human population is latently-infected with. Various immunological and molecular *in vitro* studies have been facilitated by the use of EBV's ability to infect and transform B cells to immortalized polyclonal B cell lines. Many of these studies use freshly isolated cord-blood mononuclear cells (CBMC). Some experiments may, however, require EBV infection of samples that have been prospectively collected and cryo-preserved. Here we share a protocol that we used to successfully infect B cells from cryo-preserved CBMCs and peripheral-blood mononuclear cells (PBMC) (Sohlberg *et al.*, 2013; Saghafian-Hedengren *et al.*, 2013).

Materials and Reagents

1. Cryo-preserved CBMC
2. Cryo-preserved PBMC
3. EBV strain B95-8 containing supernatant
   Note: The batch here had a titer of 2.5 x 10^5 Ramos infectious units. RaIU, which was determined by infection of the EBV negative Burkitts lymphoma B-cell line Ramos, followed by anti-complement immunoflourescent assay (ACIF) to detect the number of infected cells.
4. RPMI 1640 (Life Technologies)
5. 10% heat-inactivated fetal-calf serum (Hyclone)
6. L-glutamine (2 mmol/l)

7. penicillin G-sodium (100 U/ml)
8. streptomycin sulfate (100 mg/ml) (Merck KGaA)
9. Complete cell-culture medium (see Recipes)

Equipment

1. 48-well flat-bottomed tissue-culture treated plates (Sarstedt AG)
2. Humified incubator with for adjustment of 37 °C and 5% CO_2 for cell culture

Procedure

Note about sample handling: Gentle and fast processing of samples during isolation of mononuclear cells contributes to higher B cell viability, which is central for successful in vitro infection.

1. Thaw CBMCs or PBMCs quickly at room temperature and wash two times with incomplete RPMI-1640 at 350 - 400 *x g* and 5-10 min at room temperature.
2. Determine cell numbers, and if applicable, divide cells to fractions that will be EBV infected and those that will serve as non-infected controls.
   Note: Remember that the cell numbers and concentrations in each well should be matched for the infected and non-infected fractions.
3. Wash an additional time with RPMI 1640.
4. For non-infected control cultures: Re-suspend cells in complete cell-culture medium to a concentration of 10^6 cells/ml and allocate to the appropriate wells in the plate. Incubate at 37 °C and 5% CO_2.
5. For EBV infection, discard supernatant completely and resuspend 10^6 cells per 100 µl B95-8 virus–containing supernatant in a small tube. Incubate at 37 °C and 5% CO_2 for 90 min with gentle mixing by swirling the tube every 30 min.
6. Wash the cells once with complete cell-culture medium, discard supernatant and re-suspended in complete cell-culture medium to a concentration of 10^6 cells/ml and then allocate to the appropriate wells in the plate.
7. Feed cells with complete medium on a weekly basis. The proportion of EBV-transformed B cells will increase with time and eventually these cells take over the entire culture. As we assessed the dynamics of NK and T cell and their functional capacity (Sohlberg *et al.*, 2013; Saghafian-Hedengren *et al.*, 2013) in our previous experimental settings, we found a 1-2 week-period of time suitable for this purpose.
8. The presence of EBV-infected cells can be confirmed by immunofluorescence staining for latent membrane protein-1 and EBV nuclear Ag 2 (Rasul *et al.*, 2012).

Representative data

Refer to Figure 1 in Sohlberg *et al.* (2013) for representative data on B cell characteristics following *in vitro* EBV infection of B cells.

Notes

1. Careful and fast processing of samples during isolation of mononuclear cells contributes to higher B cell viability, which is central for successful downstream *in vitro* EBV infection.
2. This protocol uses cells from EBV seronegative donors. Deplete T cells (by for instance magnetic-based methods to remove $CD3^+$ cells from StemCell™ or Miltenyi Biotech) prior to EBV *in vitro* infection in case samples are from EBV seropositive donors.

Recipes

1. Complete cell-culture medium
   10% heat-inactivated fetal-calf serum
   L-glutamine (2 mmol/l)
   Penicillin G-sodium (100 U/ml)
   Streptomycin sulfate (100 mg/ml)

Acknowledgments

This protocol has been adapted from the previously published paper Sohlberg *et al.,* (2013). At the time of development and implementation of this protocol, the following sources of funding were used: Swedish Research Council Grants 57X-15160-07-03 and 57X-15160-10-4, the Swedish Association for Allergology, the Ragnar Söderberg Foundation, the Ellen, Walter and Lennart Hesselman Foundation, the Konsul Th. C. Bergh Foundation, the Golden Jubilee Memorial Foundation, the Petrus and Augusta Hedlund Foundation, the Crown Princess Lovisa's/Axel Tielman's Foundation, the Swedish Cancer Society, and the Karolinska Institute. E.R. and N.N. are recipients of cancer research fellowships from the Cancer Research Institute (New York)/Concern Foundation (Los Angeles). We thank Ehsan Rasul and Eva Klein (Department of Microbiology, Tumor and Cell Biology, KI, Stockholm, Sweden) for their contribution in optimizing the protocol.

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